

Appl. No. 10/019,676
Amdt. Dated December 9, 2004
Reply to Office Action of September 9, 2004

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12/30/04*

Listing of Claims:

1-72. Canceled.

73. (Previously Presented) A method for performing an immunodiagnostic test for a veterinary disease in an animal comprising:

- a) measuring a resonant frequency of a piezoelectric (Pz) crystal on which is immobilized an antigen from an infectious agent associated with the veterinary disease, or an antibody specific for an antigen from said infectious agent;
- b) contacting said crystal with a biological specimen from said animal to be tested, wherein said Pz crystal was previously used in a test which was negative for said infectious agent;
- c) measuring a resonant frequency of said crystal following step (b);
- d) comparing the resonant frequency measured in step (a) with the resonant frequency measured in step (c) wherein if the difference between the two frequencies is equal to or greater than a cut-off threshold value then said biological specimen is positive for the presence of said infectious agent.

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74. (Previously Presented) The method of claim 73, wherein said infectious agent is associated with *Salmonella enteritidis* (SE) or PRRSV disease.

75. (Previously Presented) The method of claim 73, wherein said antigen is a recombinant antigen.

76. (Previously Presented) The method of claim 73, wherein said infectious agent is a bacterium or a virus.

77. (Previously Presented) The method of claim 73, wherein following immobilization of said antigen or antibody in step (a), said crystal is contacted with a blocking reagent.

78. (Previously Presented) The method of claim 73, wherein said Pz crystal comprises an AT-cut crystalline quartz crystal.

79. (Previously Presented) The method of claim 78 wherein said Pz crystal further comprises silver or gold electrodes.

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80. (Previously Presented) The method of claim 78, wherein said Pz crystal further comprises an oscillator circuit capable of electrically stimulating said Pz crystal to oscillate at its inherent resonant frequency.

81. (Previously Presented) The method of claim 73, wherein said resonant frequencies are measured using a universal counter.

82. (Currently Amended) The method of claim 73, wherein said antigen or antibody is immobilized by a method selected from a group consisting of 1) physical adsorption onto a bare metal electrode of said crystal, 2) physical adsorption onto a metal electrode of a hydrophobic polymer modified crystal, and 3) covalent binding onto a metal electrode of a silane or thiol compound modified crystal.

83. (Previously Presented) The method of claim 73, wherein said antigen or antibody is immobilized by dipping said Pz crystal into a solution of said antigen or antibody.

84. (Previously Presented) The method of claims 73, wherein said biological specimen is diluted prior to said contacting.

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85. (Previously Presented) The method of claim 77, further comprising washing steps following step (a), the step of blocking, and step (c) wherein said washing is with a physiological buffer comprising a detergent.

86. (Previously Presented) The method of claim 85, wherein said physiological buffer is a phosphate buffered saline.

87. (Previously Presented) The method of claim 77, wherein said blocking reagent is a non-active protein.

88. (Previously Presented) The method of claim 87, wherein said blocking agent is bovine serum albumin or casein buffer.

89. (Previously Presented) The method of claim 77, wherein said blocking reagent is applied by a dip technique or a drop technique.

90. (Previously Presented) The method of claim 73, wherein said contacting with a biological specimen is performed in a liquid phase or in a vapor phase.

91. (Previously Presented) The method of claim 73, wherein said cut-off threshold is defined as the mean value of negative controls plus three times the standard deviation.

92. (Withdrawn) The method of claim 74, wherein said antigen is a transmembrane envelope protein.

93. (Withdrawn) The method of claim 92, wherein said transmembrane envelope protein is recombinantly produced as a fusion protein comprising glutathione S-transferase.

94. (Previously Presented) The method of claim 74, wherein said antigen comprises a peptide of SEQ ID NO:2.

95. (Previously Presented) A diagnostic kit for performing an immunodiagnostic test for a veterinary disease in an animal, comprising a Pz crystal on which is immobilized an antigen from an infectious agent associated with the veterinary disease, or an antibody specific for an antigen from said infectious agent, wherein said test is conducted according to the steps:

- a) measuring a resonant frequency of said Pz crystal;
- b) contacting said crystal with a biological specimen from said animal to be tested, wherein if said Pz crystal is contacted with a biological sample which is negative for said infectious agent, said crystal can be reused to perform another test;

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be tested, wherein if said Pz crystal is contacted with a biological sample which is negative for said infectious agent, said crystal can be reused to perform another test;

- c) measuring a resonant frequency of said crystal following step (b);
- d) comparing the resonant frequency measured in step (a) with the resonant frequency measured in step (c) wherein if the difference between the two frequencies is equal to or greater than a cut-off threshold value then said biological specimen is positive for the presence of said infectious agent.

96. (Previously Presented) The diagnostic kit of claim 95, wherein said antigen is a recombinant antigen.

97. (Previously Presented) The diagnostic kit of claim 95, wherein said infectious agent is associated with *Salmonella enteritidis* (SE) or PRRSV disease.

98. (Previously Presented) The diagnostic kit of claim 96, wherein said recombinant antigen comprises a protein of SEQ ID NO:2.

99. (Previously Presented) The method of claim 73, wherein the method further comprises the step of regenerating a used Pz crystal coated with i) a bound antigen or ii) a bound antibody by washing with a buffer containing boric acid/KCl-NaOH to remove any protein bound

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to said antigen or antibody while not removing said antigen or antibody thereby allowing said Pz crystal with bound antigen or antibody to be reused.

100. (Previously Presented) The method of claim 73, wherein the method further comprises the step of regenerating a used Pz crystal by washing with dichromate acid thereby removing any bound protein and any bound antigen to produce a clean crystal to which new antigen can be bound.

101. (Previously Presented) A piezoelectric (Pz) crystal coated with an antigen, or an antibody specific for said antigen, from an infectious agent associated with Salmonella enteritidis (SE) or PRRSV disease.

102. (Previously Presented) The Pz crystal of claim 101, wherein said antigen is a recombinant antigen.

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- b) contacting said crystal with a biological specimen from said animal to be tested, wherein said Pz crystal was previously used in a test which was negative for said infectious agent;
- c) measuring a resonant frequency of said crystal following step (b);
- d) comparing the resonant frequency measured in step (a) with the resonant frequency measured in step c) wherein if the difference between the two frequencies is equal to or greater than a cut-off threshold value then said biological specimen is positive for the presence of said infectious agent.

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76. (Previously Presented) The method of claim 73, wherein said infectious agent is a bacterium or a virus.

77. (Previously Presented) The method of claim 73, wherein following immobilization of said antigen or antibody in step (a), said crystal is contacted with a blocking reagent.

78. (Previously Presented) The method of claim 73, wherein said Pz crystal comprises an AT-cut crystalline quartz crystal.

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80. (Previously Presented) The method of claim 78, wherein said Pz crystal further comprises an oscillator circuit capable of electrically stimulating said Pz crystal to oscillate at its inherent resonant frequency.

81. (Previously Presented) The method of claim 73, wherein said resonant frequencies are measured using a universal counter.

82. (Previously Presented) The method of claim 73, wherein said antigen or antibody is immobilized by a method selected from a group consisting of 1) physical adsorption onto a bare metal electrode of said crystal, 2) physical adsorption onto a metal electrode of a hydrophobic polymer modified crystal, and 3) covalent binding onto a metal electrode of a silane or thiol compound modified crystal.

83. (Previously Presented) The method of claim 73, wherein said antigen or antibody is immobilized by dipping said Pz crystal into a solution of said antigen or antibody.

84. (Previously Presented) The method of claims 73, wherein said biological specimen is diluted prior to said contacting.

85. (Previously Presented) The method of claim 77, further comprising washing steps following step (a), the step of blocking, and step c) wherein said washing is with a physiological buffer comprising a detergent.

86. (Previously Presented) The method of claim 85, wherein said physiological buffer is a phosphate buffered saline.

87. (Previously Presented) The method of claim 77, wherein said blocking reagent is a non-active protein.

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89. (Previously Presented) The method of claim 77, wherein said blocking reagent is applied by a dip technique or a drop technique.

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92. (Withdrawn) The method of claim 74, wherein said antigen is a transmembrane envelope protein.

93. (Withdrawn) The method of claim 92, wherein said transmembrane envelope protein is recombinantly produced as a fusion protein comprising glutathione S-transferase.

94. (Previously Presented) The method of claim 74, wherein said antigen comprises a peptide of SEQ ID NO:2.

95. (Previously Presented) A diagnostic kit for performing an immunodiagnostic test for a veterinary disease in an animal, comprising a Pz crystal on which is immobilized an antigen from an infectious agent associated with the veterinary disease, or an antibody specific for an antigen from said infectious agent, wherein said test is conducted according to the steps:

- a) measuring a resonant frequency of said Pz crystal;
- b) contacting said crystal with a biological specimen from said animal to be tested, wherein if said Pz crystal is contacted with a biological sample which is negative for said infectious agent, said crystal can be reused to perform another test;

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c) measuring a resonant frequency of said crystal following step (b);
d) comparing the resonant frequency measured in step (a) with the resonant frequency measured in step c) wherein if the difference between the two frequencies is equal to or greater than a cut-off threshold value then said biological specimen is positive for the presence of said infectious agent.

96. (Previously Presented) The diagnostic kit of claim 95, wherein said antigen is a recombinant antigen.

97. (Previously Presented) The diagnostic kit of claim 95, wherein said infectious agent is associated with *Salmonella enteritidis* (SE) or PRRSV disease.

98. (Previously Presented) The diagnostic kit of claim 96, wherein said recombinant antigen comprises a protein of SEQ ID NO:2.

99. (Previously Presented) The method of claim 73, wherein the method further comprises the step of regenerating a used Pz crystal coated with i) a bound antigen or ii) a bound antibody by washing with a buffer containing boric acid/KCl-NaOH to remove any protein bound to said antigen or antibody while not removing said antigen or antibody thereby allowing said Pz crystal with bound antigen or antibody to be reused.

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100. (Previously Presented) The method of claim 73, wherein the method further comprises the step of regenerating a used Pz crystal by washing with dichromate acid thereby removing any bound protein and any bound antigen to produce a clean crystal to which new antigen can be bound.

101. (Cancelled) A piezoelectric (Pz) crystal coated with an antigen, or an antibody specific for said antigen, from an infectious agent associated with Salmonella enteritidis (SE) or PRRSV disease.

102. (Cancelled) The Pz crystal of claim 101, wherein said antigen is a recombinant antigen.